치의학박사 학위논문

Effect of 1,440-nm Nd:YAG Laser Irradiation on Pain and Neuropeptide/Cytokine Reduction from the Teeth with Persistent Apical Periodontitis

1440-nm Nd:YAG 레이저가 지속적인 치근단 치주염을 가지는 치아의 동통 및 뉴로펩타이드/싸이토카인 감소에 미치는 영향에 대한 연구

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유 연 지

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Abstract

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Introduction

The purpose of this study was to investigate the efficacy of a 1,440-nm Nd:YAG laser on relieving pain in relation to the levels of neuropeptides and inflammatory cytokine in the root canal exudates of the teeth with persistent symptomatic apical periodontitis.

Materials and methods

Forty patients referred for further management and treatment of the teeth with

persistent symptomatic apical periodontitis were randomly assigned to treatment groups: group L, intracanal irradiation of 1,440-nm Nd:YAG laser with a 300-μm-diameter fiber-optic tip in addition to conventional root canal re-treatment; group C, conventional root canal re-treatment. The degrees of both spontaneous pain and pain on percussion before and after treatment were recorded (VAS; visual analogue scale), and root canal exudate samples were collected to quantify the associated levels of substance P (SP), calcitonin gene-related peptide (CGRP), and matrix metalloproteinase (MMP)-8 by immunoassay.

The effect of experimental treatment procedures on pain relief (VAS scores) and the changes in SP, CGRP, and MMP-8 concentration (%), and the differences among experimental treatment groups were analyzed. The correlations between the degree of perceived pain (VAS scores of spontaneous pain and pain on percussion) and the levels of SP, CGRP, and MMP-8 in the root canal exudates were analyzed as well. The level of significance was set at 5%.

Results

All of the measured parameters were significantly reduced in group L ($p < .05$), while the levels of pain on percussion, CGRP and MMP-8 were significantly reduced in group C ($p < .05$). The 1,440-nm Nd:YAG laser had significantly better effect on the relief of pain on percussion and the reduction of SP, CGRP and MMP-8 levels in root canal exudate. The VAS scores of perceived pain correlated with pain-related neuropeptides and inflammatory cytokine levels in root canal exudates. SP levels correlated directly with CGRP levels ($p < .05$).

Conclusions

The 1,440-nm Nd:YAG laser irradiation via fiber-optic tip to the teeth with persistent apical periodontitis provided promising consequences of pain and inflammation modulation.

Key words: Nd:YAG laser, neuropeptides, pain, visual analogue scale, fiber-optic tip

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Introduction

A patient undergoing endodontic treatment suffers from varying levels of pain typically expressed as “spontaneous pain” or “pain on percussion” at unpredictable point of treatment period. Once the source of inflammation is removed from the root canal system under the proper treatment modality, the associated pain may decrease in intensity or disappear. Thus, the degree of pain expressed by the patient provides clinical confirmation to estimate whether a certain treatment procedure reduced the severity of periapical inflammation. However, the level of pain per se cannot be used as a quantitative diagnostic criterion of the degree of inflammation. In this regard, there have been attempts to visualize the clinical effect of endodontic treatment procedures on pain relief in relation to the levels of pro-inflammatory cytokines and bacterial endotoxins (1-3).

Various techniques have been used for complete debridement and decontamination of the root canal system in attempts to reduce the pain and inflammation associated with endodontic treatment procedures (4-6). Lasers were introduced in endodontics to overcome the limitations of those currently used techniques caused by the inherent anatomic complexity of the root canal system. Most studies (7, 8) used near-infrared lasers such as diode and 1,064-nm neodymium-doped:yttrium-aluminum-garnet (Nd:YAG) lasers, medium-infrared erbium-family lasers, or far-infrared CO_2 lasers (10,500-nm); and they have confirmed the effectiveness of these lasers in root canal disinfection when used in conjunction with root canal irrigants such as sodium

hypochlorite (NaOCl) or ethylenediaminetetraacetic acid (EDTA). Given that near-infrared lasers have no ablative effect on hard tissue while median-infrared lasers have undesirable ablative and thermal effects on dentinal walls, current efforts to maximize the use of lasers in endodontics are focused on finding the optimal wavelength and appropriate lasing method.

Recently, a new device which delivers high-wavelength near-infrared Nd:YAG laser using flexible fiber-optic tip has been introduced and Moon et al. (9) reported the effectiveness of this 1,320-nm Nd:YAG laser when used with a fiber-optic tip for smear layer removal. Consequently, it could be assumed that high-wavelength Nd:YAG laser (1,440-nm) may have more diverse effects in relieving pain and alleviating inflammation if irradiated directly to the apical region of the root canal with a flexible fiber-optic tip. However, little is known about the effect of 1,440-nm Nd:YAG laser in endodontics in regard to the constituents of root canal exudates and the level of pain experienced by the patient, in particular.

Therefore, the purpose of this randomized prospective clinical trial was to investigate the effect of 1,440-nm Nd:YAG laser on reducing pain of the teeth with persistent symptomatic apical periodontitis focused on the pain-related neuropeptides and inflammatory cytokine levels in root canal exudates.

Materials and methods

Patient Selection

The study protocol was approved by the Institutional Review Board (IRB No.CDE12002) of Seoul National University Dental Hospital (SNUDH). Patients referred to the Department of Conservative Dentistry of SNUDH for further management and treatment of the teeth with persistent symptomatic apical periodontitis were evaluated as possible candidates for this study. Patients who have the teeth with symptomatic apical periodontitis that had previously been initiated primary root canal treatment, with no sinus tract or swelling, no contributory medical history, and no previous systemic administration of antibiotics or analgesics within 2 week of enrollment were accepted for the present clinical trial. The patient was excluded if he/she required antibiotic prophylaxis or had uncontrolled diabetes, generalized periodontitis, or if the tooth had periodontal probing depth of more than 3 mm. The teeth with periapical radiolucencies which had typical radiographic characteristics of periapical cyst (well-demarcated corticated margins with more than 10 mm in diameter, evidently showing displacement of adjacent structures or expansion of the outer cortical boundaries of the jaw) were also excluded.

For the routine root canal re-treatment procedure, the tooth was isolated with a rubber dam and the access cavity was refined. If necessary, topical anesthetic agent was applied to prevent immediate increase of substance P release as a result of needle injection. If the

symptom was too severe to place the clamp even with the aid of topical anesthesia, the whole quadrant was isolated and the clamp was placed on another tooth (usually the most distal tooth of the isolated quadrant).

Upon access, teeth with confirmed crack line under microscopic evaluation were excluded from the study. The operator carefully removed previous root canal filling material if present, confirmed patency of the root canals with ISO #10 K-files, and determined the working length using an electronic apex locator (Dentaport ZX II, Morita Co., Kyoto, Japan) and radiographs. The root canals were then enlarged to 3 size larger than the initial apical file, at least up to size ISO #35, using nickel-titanium rotary instruments (ProFile, Dentsply Maillefer, Bellaigues, Switzerland), gates glidden burs, stainless steel K-files, and RC Prep (Premier Dental Products, Norristown, PA, USA). All instrumentations were accompanied by careful and copious irrigation with a sufficient volume of 3.5% NaOCl. Then, the root canals were dried with sterile paper points. The root canals were filled with calcium hydroxide (Metapaste, Meta Biomed, Osong, Korea) and temporarily sealed the access cavity with temporary filling material (Caviton, GC, Tokyo, Japan).

Candidates were recalled 1 week later and assessed for clinical signs and symptoms. If there were no sign of clinical symptoms, the root canals were filled and the patients were excluded from the study. If the symptom persisted, the patients were asked whether to participate the present study or not. When the patient agreed to participate, he/she was subjected as a participant and written and verbal informed consent was acquired.

The power analysis was conducted on the basis of the minimum clinically significant difference in the visual analog scale (VAS) score. A sample size of 16 patients in each

group was calculated to be sufficient to detect clinically important differences of VAS score (alpha at level 0.05, 90% power, and effect size of 1.2). Ultimately, a sample size of 20 patients in each group was determined, considering the dropout rate such as loss to follow-up. All accepted patients were randomly assigned to treatment groups as follows: group L, laser application in addition to conventional root canal re-treatment; group C, conventional root canal re-treatment.

Experimental Treatment Procedures and Sample Collection

Each patient's pain level (spontaneous pain and pain on percussion test) was recorded before the initiation of each experimental treatment procedure using the VAS score (0–10 scale: 0, no pain; 10, extremely severe pain). For percussion test, the cusps of each tooth were percussed 3 times using the shaft of periodontal probe. First, the normal asymptomatic tooth was tested to make the patient experience the normal tapped feeling of the percussion test and to rule out the possible false-positive response. Then, the subjected tooth and its adjacent teeth were tested in a randomized order, and the pain of the subjected tooth that the patient perceived was recorded using VAS scores.

The tooth was re-accessed under rubber dam isolation and the root canals were gently irrigated with sterile saline, using a 25 G side-vent needle. After the gross irrigant was removed by aspiration, sterile paper points were inserted into the root canals and left in place for 2 minutes to collect the root canal exudate. The paper points soaked with root canal exudate were placed into a 1.5 mL Eppendorf tube containing 100 μL of Tris-HCl buffer, pH 7.5 with 0.15 mol/L NaCl and 1 mmol/L $CaCl_2$. The tubes were placed on a

shaker at room temperature for 3 hours and then stored at -70℃ for further analysis.

The operator re-checked the patency and working length. Copious root canal irrigation with sufficient volume of 3.5 % sodium hypochlorite was performed using 27 G side vent needle. Then, the root canals were dried with sterile paper points. For the experimental treatment procedure in group L, the Nd-YAG laser (Slimlift MPX; B&B Systems, Seoul, Korea) was applied for 10 seconds to the apical 3-mm level of the root canals with 300-μm in diameter fiber-optic tip. The laser was set to 1,440-nm wavelength, 200 mJ/cm^2 energy and 1 Hz frequency. If the patient was allocated to the group C, the fiber-optic tip of the laser was focused on the other side of the rubber dam sheet, mimicking laser irradiation sound to prevent placebo effect. The root canals were filled with calcium hydroxide and the access cavity was sealed with temporary filling material. The participants were provided escape medication (Ibuprofen / Arginine) in case of flare up with severe pain and instructed for emergency visit.

The patient was recalled 3 days after the experimental treatment procedure for the post-treatment evaluation. Post-treatment pain levels were recorded (VAS), and root canal exudates were collected again as described above. After root canal exudate sample collection, patients received routine root canal treatment procedure. Subsequently, all canals were filled with gutta-percha and root canal sealers.

Substance P and Calcitonin Gene-related Peptide Analyses

Quantification of neuropeptides was completed with immunoassay kits according to the manufacturers' instructions; SP, enzyme-linked immunosorbent assay kit (Parameter,

Human substance P assay; R&D Systems Inc., Minneapolis, MN, USA) and calcitonin gene-related peptide (CGRP), EIA kit (Human CGRP enzyme immunoassay kit; SPUbio, Massy, France). Fifty microliters of each sample, standards, and controls were pipetted into 96-well plates coated with specific antibodies. All samples were analyzed in duplicate. The absorbency of each sample at 450 nm (OD_{450}) was read using a microplate reader (Model 680; Bio-Rad, Hercules, CA, USA) and analyzed with Microplate Manager (version 5.2.1; Bio-Rad). The data were expressed as the total amount (ng) per sample.

Matrix Metalloproteinase (MMP)-8 Analysis

Matrix metalloproteinase (MMP)-8 was assayed with an assay kit from Millipore (Human sepsis magnetic bead panel 2; Billerica, MA, USA). All specimens were assayed in duplicate according to the manufacturer's protocols. Multiplex immunoassays were performed using the Luminex 100 IS System (Luminex Corp., Austin, TX, USA). The standard curve for sample cytokine concentration determination was used. MMP-8 concentrations were calculated based on the basis of the standard curves using Bio-Plex Manager 6.1 (Bio-Rad). The data were expressed as the total amount (ng) per sample.

Statistical analysis

The effect of experimental treatment procedures on pain relief and the changes in SP, CGRP and MMP-8 concentrations were analyzed using a Wilcoxon signed rank sum test.

The differences among experimental treatment groups were analyzed using a nonparametric Kruskal-Wallis analysis. A series of Mann-Whitney tests were used for multiple comparisons. Spearman correlation coefficients were calculated to determine the correlation between the degree of perceived pain (VAS scores for spontaneous pain and pain on percussion) and the levels of neuropeptides and inflammatory cytokine in the root canal exudates. The level of significance was set at 5%.

Results

Forty teeth from forty patients (16 men and 24 women) were enrolled in this study. None of the participants were lost to follow-up, and each of them did not take any of the escape medication. The preoperative demographic features and pain characteristics are presented in Table 1. The preliminary statistical analysis confirmed no evidence of selection bias in terms of treatment group assignment.

Pretreatment versus post-treatment levels of pain are shown in Figure 1, and the changes in the SP, CGRP, and MMP-8 concentrations before and after the treatments are demonstrated in Figure 2. The medians, mean values, and standard deviations of the VAS score changes of pain perceived by the patient and the concentration changes of the neuropeptides and inflammatory cytokine in the root canal exudates before and after the treatment are summarized in Table 2.

The Wilcoxon signed rank sum test showed that additional laser application (group L) significantly reduced spontaneous pain, pain on percussion, and the levels of SP, CGRP, and MMP-8 ($p < .05$), whereas conventional root canal treatment procedure (group C) significantly reduced the degree of pain on percussion as well as CGRP and MMP-8 levels ($p < .05$).

Comparing the intergroup differences, the 1,440-nm Nd:YAG laser irradiation was significantly more effective in reducing pain on percussion ($p = .003$) and in decreasing SP ($p = .002$), CGRP ($p = .049$) and MMP-8 ($p = .002$) concentrations.

The results of correlation analysis are demonstrated in Figure 3. The VAS scores of

spontaneous pain were positively correlated with SP (r_s = .303, p = .006) and MMP-8 (r_s = .405, p = .000) levels, and those of pain on percussion were positively correlated with SP (r_s = .247, p = .027), CGRP (r_s = .431, p = .000), and MMP-8 (r_s = .222, p = .047) levels. SP levels correlated directly with CGRP levels (r_s = .405, p = .000).

Discussion

Root canal treatment is a procedure to remove the source of inflammation causing pulpitis or apical periodontitis that is accompanied by undesirable occurrences such as pain or other clinical signs and symptoms. Unfortunately, varying levels of pain and discomfort may persist even after removal of inflammation source by cleaning and shaping procedures. Except for the case of nonodontogenic origin (10), such pain is often caused by remaining intracanal inflammation, pulpal inflammation extended via apical foramen beyond periodontal ligament (PDL) and/or periapical tissue, or newly induced inflammation resulting from biomechanical instrumentation and chemical debridement. This type of pain is not a rare phenomenon and occurs in 3%–58% of cases with various severities (10-14). A number of materials and techniques have been investigated as part of efforts to manage symptomatic apical periodontitis during endodontic treatment. In this regard, we evaluated the therapeutic effect of 1,440-nm Nd:YAG laser energy delivered by a 300-μm fiber-optic tip directly to the apical area, expecting anti-inflammatory and pain-relieving actions (15-17). In fact, VAS scores for spontaneous pain and pain on percussion which serve as markers for the subjective expression of pain were reduced in certain degrees after the treatment in this study.

As a qualitative measure of subjective pain, VAS is a suitable tool in many clinical studies for its ease of understanding, high reproducibility, and being unaffected by gender (18). However, the VAS is a ranking scale, rather than a tool for quantifying the degree of pain. Therefore, the results cannot be interpreted strictly using parametric statistical

analysis (calculating mean or standard deviation), regardless of the number of subjects. In this study, the perceived pain level decreased after treatment, but the pain-related molecule levels in root canal exudate did not reach “0” in even pain-free patients. There are no set criteria for deducing the severity of pain or inflammation from the levels of neuropeptides or inflammatory cytokines, because each patient has a different pre-operative status with a unique pain threshold and immune system. Consequently, there have been approaches to quantify the degree of pain or inflammation in accordance with neuropeptides or inflammatory cytokines from PDLs of extracted teeth (19, 20), root canal exudates (2, 3, 21) and gingival crevicular fluids (22, 23) of the painful teeth. Notably, root canal exudate, mixture of molecules originated from PDL and/or periapical area diffused through the apical foramen, is a rather non-invasive and relevant diagnostic tool to indicate and monitor the degree of inflammation and/or endodontic pain.

During the inflammatory process of pulpal and periapical tissue complex, the destruction of PDL is initiated with the degradation of the extracellular matrix (ECM) by enzymes like MMPs, which in turn accelerates further inflammatory or immune reactions. Among the well-known MMPs, MMP-8 (collagenase-2) is partly related to the severity of clinical symptoms (24). Wahlgren et al. (3) reported MMP-8 levels to be a potential diagnostic tool for use when evaluating the status of periapical inflammation, and Shin et al. (23) also showed a correlation between MMP-8 levels and the degree of pain reported by the patient. Furthermore, it has been demonstrated that PDL inflammation has a neurogenic component; nerve fibers control vascular tone and immune response through the actions of neuropeptides such as SP and CGRP (25). Those neuropeptides are considered major determinants of the inflammatory process in periapical tissues (26), and

they are frequently co-localized (27). These molecules are produced and released from sensory nerve fibers of the PDL during inflammatory and immune processes; and their elevated levels sustain the vicious circle that underlies inflammation (28), causing nerve fiber sensitization, spontaneous depolarization, and enhancing pain response. These biological effects are observed during persistent symptomatic apical periodontitis (20, 29). In this sense, it was another purpose of this study to speculate whether laser-induced pain relief effect was correlated with changes in MMP-8 and neuropeptide concentrations.

In this study, the patients who received additional laser treatment showed more predictable relief of pain, particularly in pain on percussion compared to conventional root canal therapy via $Ca(OH)_2$ intracanal medication procedure. The severity of pain on percussion is known to be directly proportional to PDL inflammation (20, 29). Considering the relatively higher correlation of CGRP concentration with pain on percussion rather than spontaneous pain, it can be presumed that 1,440-nm Nd:YAG laser may have certain extent of sedative effect on peripheral C-fibers in the PDL, thus providing therapeutic effect to teeth with symptomatic apical periodontitis. However, it should not to be overlooked that this phenomenon is partly assumed to be the result of statistic skewness. All of the subjected root canals were enlarged with confirmed patency and working length, and had been medicated with $Ca(OH)_2$. Therefore, the overall incidence of spontaneous pain was relatively lower (35%) than that of percussion pain (100%), spawned small calculated effect size. The influence of laser irradiation on spontaneous pain remains to be investigated further.

Lasers affects the target tissues through photothermal and photomechanical effects (30). Thus, we elected to use Nd:YAG laser, expecting advantages of the near-infrared laser

over medium-infrared laser, and chose to use a higher wavelength (1,440-nm rather than 1,064-nm) because the amount of emitted photon is proportional to wavelength. Interestingly, it can be deduced from the present result that the lased energy directly delivered to the apical area might have provided not a photomechanical or photoacoustic mechanism, but rather a photochemical effect (31), affecting the root canal exudate constituent of symptom-related molecules. The confirmed changes in concentration of symptom-related molecules suggest the plausible rationale of this laser on modulation of the pain / inflammation / immune nexus in molecular levels.

The concern regarding lasing inside the root canal is a possibility of thermal tissue damage (32). To minimize this undesirable side effect, previous studies suggest circular movement of the tip inside the root canal (33) or retracting the tip in helical movement (34), in conjunction with root canal irrigants. However, we used relatively lower power setting (200 mJ/pulse and 1 Hz; average power, 0.2 W) in a dry canal, maintaining the fiber-optic tip 3-mm short of working length. Previous research (35) has shown that the Nd:YAG laser irradiation (1,064-nm, 1.5 W) in a dry root canal did not increase the temperature of surrounding tissue more than 10℃, which is the threshold for the occurrence of evident bone tissue damage (36). The low repetition rate pulsing method used in this study also allowed sufficient time for thermal relief between pulses. Therefore, the power setting in the current study can be considered as safe. Furthermore, the use of static irradiation could prevent unwanted contact of the fiber-optic tip with the dentinal wall, which can cause burns in dry canal, providing predictability of the amount of delivered energy.

In conclusion, this study demonstrates favorable effects of the fiber-optic delivered

1,440-nm Nd:YAG laser on the pain and inflammation modulation suggesting a causal basis for use in non-surgical endodontics. The ability of this laser to modulate endodontic pain opens up various possibilities on its effects on peripheral nociceptive mediators.

References

1. Jacinto RC, Gomes BP, Shah HN, Ferraz CC, Zaia AA, Souza-Filho FJ. Quantification of endotoxins in necrotic root canals from symptomatic and asymptomatic teeth. J Med Microbiol 2005;54:777-83.
2. Alptekin NO, Ari H, Haliloglu S, Alptekin T, Serpek B, Ataoglu T. The effect of endodontic therapy on periapical exudate neutrophil elastase and prostaglandin-E2 levels. J Endod 2005;31:791-5.
3. Wahlgren J, Salo T, Teronen O, Luoto H, Sorsa T, Tjaderhane L. Matrix metalloproteinase-8 (MMP-8) in pulpal and periapical inflammation and periapical root-canal exudates. Int Endod J 2002;35:897-904.
4. Siqueira JF, Jr., Rocas IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. J Endod 2008;34:1291-301.
5. Soukos NS, Chen PS, Morris JT, Ruggiero K, Abernethy AD, Som S, et al. Photodynamic therapy for endodontic disinfection. J Endod 2006;32:979-84.
6. Garcez AS, Ribeiro MS, Tegos GP, Nunez SC, Jorge AO, Hamblin MR. Antimicrobial photodynamic therapy combined with conventional endodontic treatment to eliminate root canal biofilm infection. Lasers Surg Med 2007;39:59-66.
7. Levy G. Cleaning and shaping the root canal with a Nd:YAG laser beam: a comparative study. J Endod 1992;18:123-7.
8. Levy G, Rizoiu I, Friedman S, Lam H. Pressure waves in root canals induced by Nd: YAG laser. J Endod 1996;22:81-4.

9. Moon YM, Kim HC, Bae KS, Baek SH, Shon WJ, Lee W. Effect of laser-activated irrigation of 1320-nanometer Nd:YAG laser on sealer penetration in curved root canals. J Endod 2012;38:531-5.

10. Nixdorf DR, Moana-Filho EJ, Law AS, McGuire LA, Hodges JS, John MT. Frequency of nonodontogenic pain after endodontic therapy: a systematic review and meta-analysis. J Endod 2010;36:1494-8.

11. Nixdorf DR, Moana-Filho EJ, Law AS, McGuire LA, Hodges JS, John MT. Frequency of persistent tooth pain after root canal therapy: a systematic review and meta-analysis. J Endod 2010;36:224-30.

12. Gondim E, Jr., Setzer FC, Dos Carmo CB, Kim S. Postoperative pain after the application of two different irrigation devices in a prospective randomized clinical trial. J Endod 2010;36:1295-301.

13. Siqueira JF, Jr., Rocas IN, Favieri A, Machado AG, Gahyva SM, Oliveira JC, et al. Incidence of postoperative pain after intracanal procedures based on an antimicrobial strategy. J Endod 2002;28:457-60.

14. Sathorn C, Parashos P, Messer H. The prevalence of postoperative pain and flare-up in single- and multiple-visit endodontic treatment: a systematic review. Int Endod J 2008;41:91-9.

15. Kreisler MB, Haj HA, Noroozi N, Willershausen B. Efficacy of low level laser therapy in reducing postoperative pain after endodontic surgery - a randomized double blind clinical study. Int J Oral Maxillofac Surg 2004;33:38-41.

16. Gomez C, Dominguez A, Garcia-Kass AI, Garcia-Nunez JA. Adjunctive Nd:YAG laser application in chronic periodontitis: clinical, immunological, and microbiological

aspects. Lasers Med Sci 2011;26:453-63.

17. Silva LA, Novaes AB, Jr., de Oliveira RR, Nelson-Filho P, Santamaria M, Jr., Silva RA. Antimicrobial photodynamic therapy for the treatment of teeth with apical periodontitis: a histopathological evaluation. J Endod 2012;38:360-6.

18. Goddard G, Karibe H, McNeill C. Reproducibility of visual analog scale (VAS) pain scores to mechanical pressure. Cranio 2004;22:250-6.

19. Caviedes-Bucheli J, Moreno JO, Carreno CP, Delgado R, Garcia DJ, Solano J, et al. The effect of single-file reciprocating systems on Substance P and Calcitonin gene-related peptide expression in human periodontal ligament. Int Endod J 2013;46:419-26.

20. Caviedes-Bucheli J, Azuero-Holguin MM, Gutierrez-Sanchez L, Higuerey-Bermudez F, Pereira-Nava V, Lombana N, et al. The effect of three different rotary instrumentation systems on substance P and calcitonin gene-related peptide expression in human periodontal ligament. J Endod 2010;36:1938-42.

21. Shahriari S, Rezaei A, Jalalzadeh SM, Mani K, Zamani A. Effect of Ibuprofen on IL-1beta, TNF-alpha and PGE2 levels in periapical exudates: a double blinded clinical trial. Iran J Immunol 2011;8:176-82.

22. Kato J, Tanne K, Ichikawa H, Matsuo S, Wakisaka S, Akai M, et al. Distribution of calcitonin gene-related peptide and substance P-immunoreactive nerve fibers and their correlation in the periodontal ligament of the mouse incisor. Acta Anat (Basel) 1992;145:101-5.

23. Shin SJ, Lee W, Lee JI, Baek SH, Kum KY, Shon WJ, et al. Matrix metalloproteinase-8 and substance P levels in gingival crevicular fluid during endodontic treatment of painful, nonvital teeth. Oral Surg Oral Med Oral Pathol Oral Radiol Endod

2011;112:548-54.

24. Reynaud af Geijersstam A, Sorsa T, Stackelberg S, Tervahartiala T, Haapasalo M. Effect of E. faecalis on the release of serine proteases elastase and cathepsin G, and collagenase-2 (MMP-8) by human polymorphonuclear leukocytes (PMNs). Int Endod J 2005;38:667-77.

25. Stashenko P, Teles R, D'Souza R. Periapical inflammatory responses and their modulation. Crit Rev Oral Biol Med 1998;9:498-521.

26. Richardson JD, Vasko MR. Cellular mechanisms of neurogenic inflammation. J Pharmacol Exp Ther 2002;302:839-45.

27. Lundy FT, Linden GJ. Neuropeptides and Neurogenic Mechanisms in Oral and Periodontal Inflammation. Crit Rev Oral Biol Med 2004;15:82-98.

28. Sacerdote P, Levrini L. Peripheral mechanisms of dental pain: the role of substance P. Mediators Inflamm 2012;2012:1-6.

29. Caviedes-Bucheli J, Azuero-Holguin MM, Correa-Ortiz JA, Aguilar-Mora MV, Pedroza-Flores JD, Ulate E, et al. Effect of experimentally induced occlusal trauma on substance p expression in human dental pulp and periodontal ligament. J Endod 2011;37:627-30.

30. Thomsen S. Pathologic analysis of photothermal and photomechanical effects of laser-tissue interactions. Photochem Photobiol 1991;53:825-35.

31. Huang YY, Sharma SK, Carroll J, Hamblin MR. Biphasic dose response in low level light therapy - an update. Dose Response 2011;9:602-18.

32. Kaitsas V, Signore A, Fonzi L, Benedicenti S, Barone M. Effects of Nd: YAG laser irradiation on the root canal wall dentin of human teeth: a SEM study. Bull Group Int

Rech Sci Stomatol Odontol 2001;43:87-92.

33. Camargo SE, Valera MC, Camargo CH, Fonseca MB, Menezes MM. Effects of Nd:YAG laser irradiation on root canal dentin wall: a scanning electron microscopic study. Photomed Laser Surg 2005;23:399-404.

34. Mohammadi Z. Laser applications in endodontics: an update review. Int Dent J 2009;59:35-46.

35. Strakas D, Franzen R, Kallis A, Vanweersch L, Gutknecht N. A comparative study of temperature elevation on human teeth root surfaces during Nd:YAG laser irradiation in root canals. Lasers Med Sci 2013;28:1441-4.

36. Eriksson AR, Albrektsson T. Temperature threshold levels for heat-induced bone tissue injury: a vital-microscopic study in the rabbit. J Prosthet Dent 1983;50:101-7.

Tables and Figures

Table 1. Demographic features and preoperative characteristics of each treatment group.

		Group L	Group C	P value
Age (yr)		44.3 ± 9.1	46.4 ± 11.3	.913
Gender	Male	9	7	.524
	Female	11	13	
Jaw	Upper	11	14	.333
	Lower	9	6	
Tooth type	Anterior	8	6	.513
	Posterior	12	14	
Spontaneous pain		8	6	.698
Pain on percussion test		20	20	.602

Group L, laser application in addition to conventional root canal re-treatment; group C, conventional root canal re-treatment.

Table 2. Medians, mean values, and standard deviations (SD) of the VAS score changes of pain perceived by the patient and concentration changes (%) of the neuropeptides and inflammatory cytokine in the root canal exudate before and after the experimental treatments.

			Median	**Mean**	**SD**
Group L	VAS score changes	Spontaneous pain	0	−1.1	1.59
		Pain on percussion	−2.5	−2.8	1.54
	Concentration changes (%)	SP	−12.42	−14.22	12.38
		CGRP	−38.29	−29.88	61.03
		MMP-8	−76.14	−68.62	27.93
Group C	VAS score changes	Spontaneous pain	0	−0.15	0.49
		Pain on percussion	−1.0	−1.4	1.39
	Concentration changes (%)	SP	−1.10	5.41	32.47
		CGRP	−20.27	−10.80	121.48
		MMP-8	−22.48	−32.73	38.55

Group L, laser application in addition to conventional root canal re-treatment; group C, conventional root canal re-treatment; VAS, visual analogue scale; SP, substance P; CGRP, calcitonin gene-related peptide; MMP, matrix metalloproteinase.

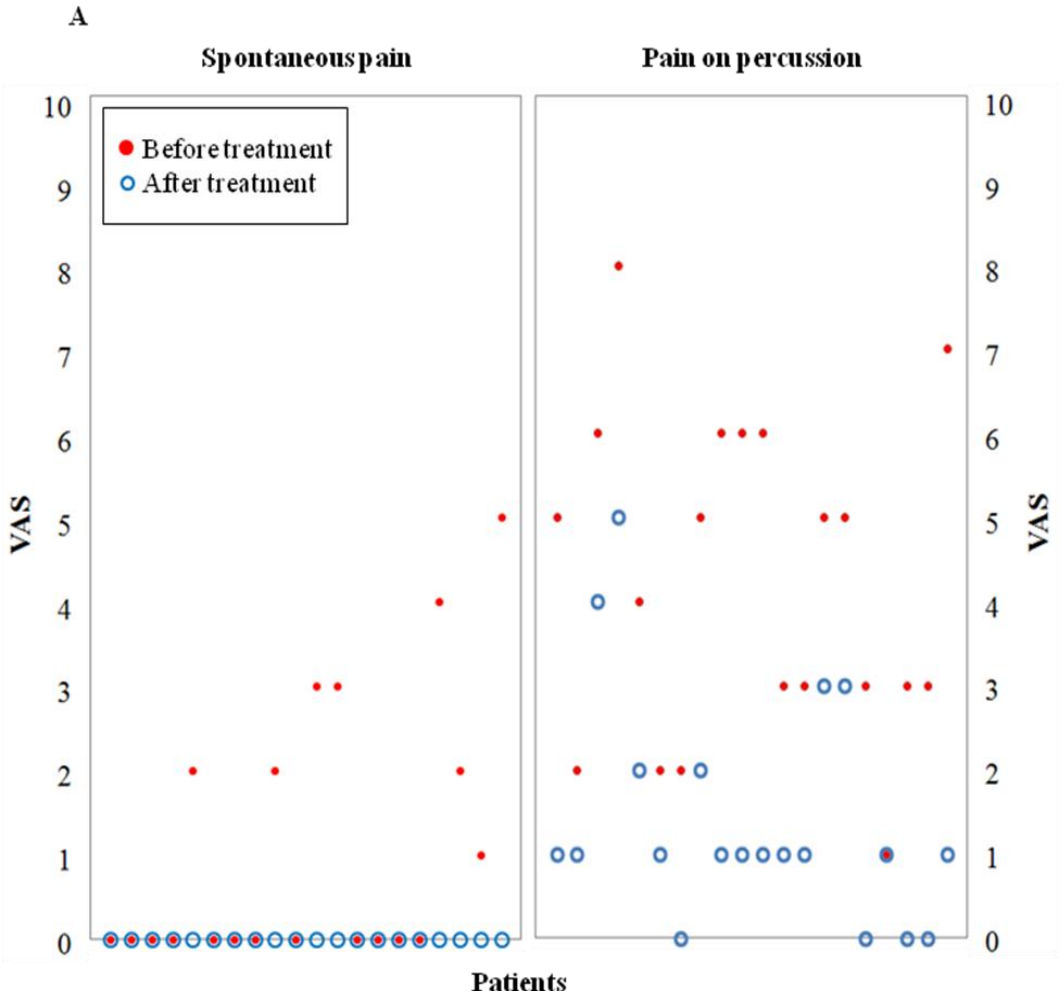


Figure 1. Preoperative and postoperative pain levels of each patient. VAS; visual analogue scale (0, no pain; 10, extremely severe pain). (A) Group L. (B) Group C.

(continued)

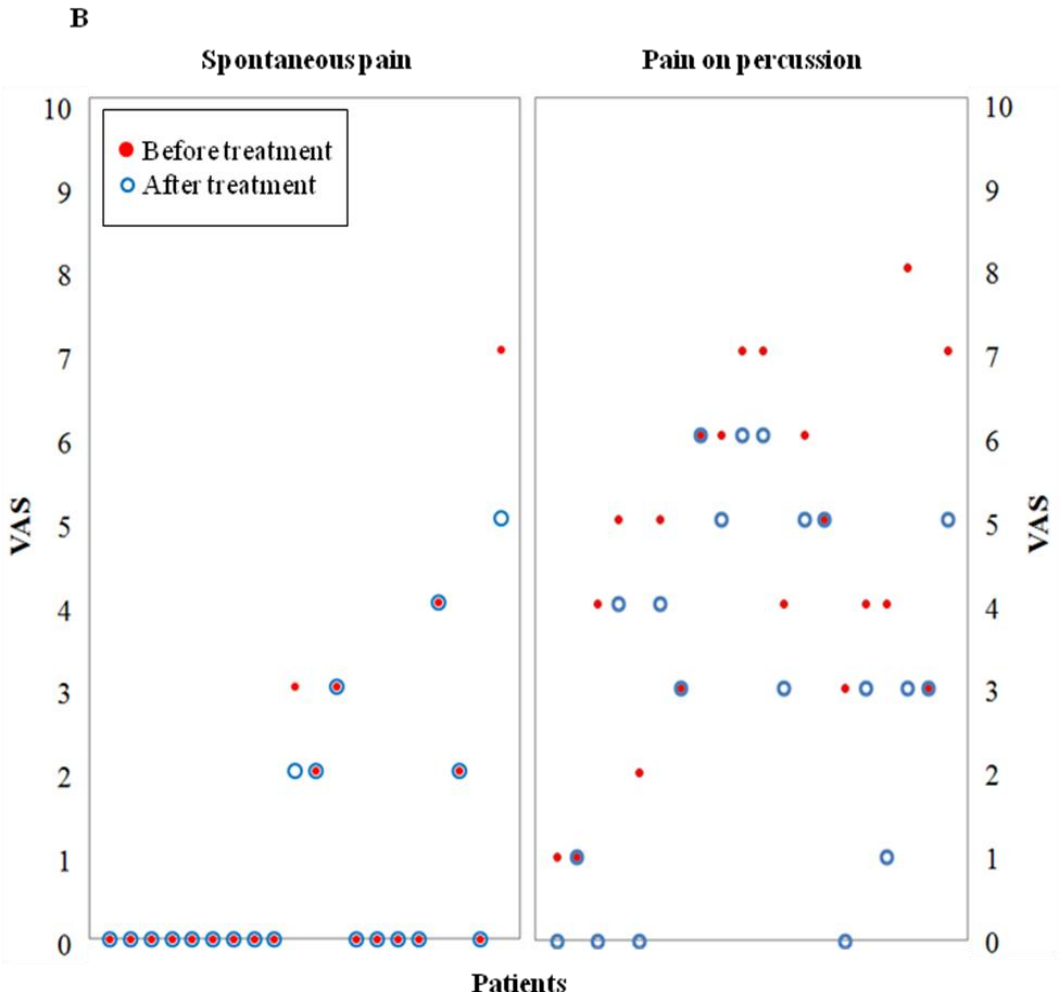


Figure 1. Preoperative and postoperative pain levels of each patient. VAS; visual analogue scale (0, no pain; 10, extremely severe pain). (A) Group L. (B) Group C.

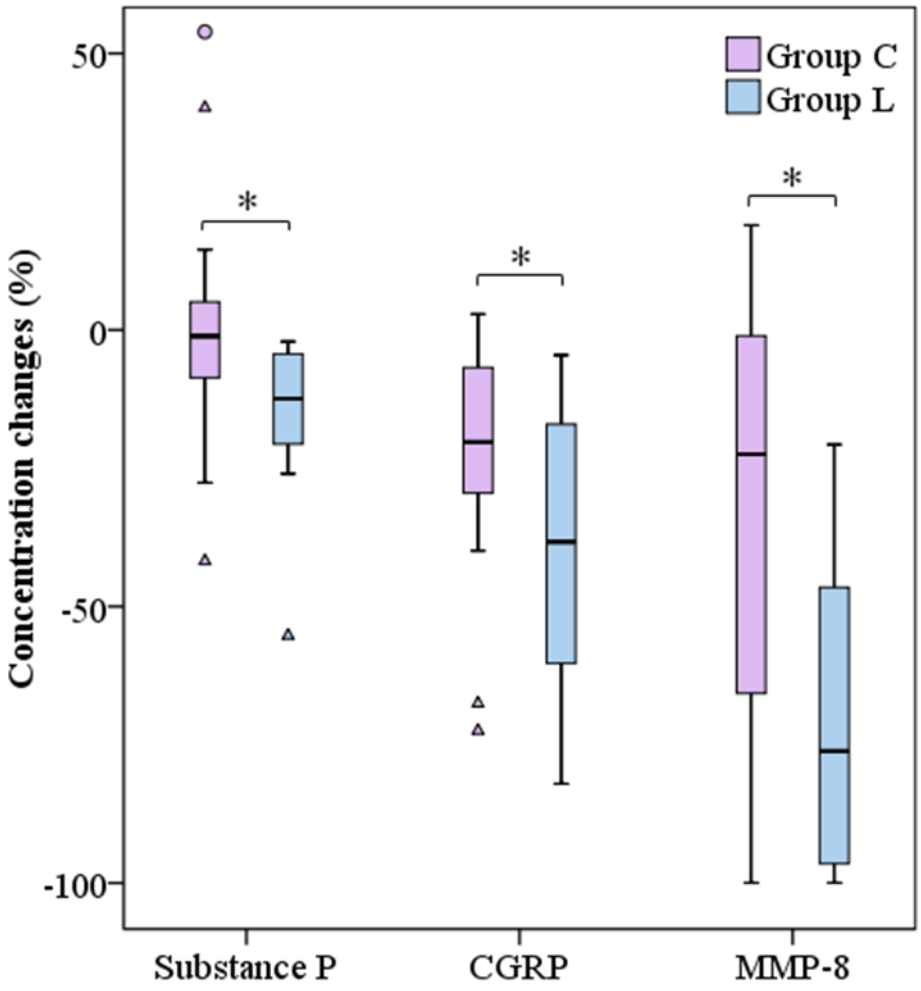
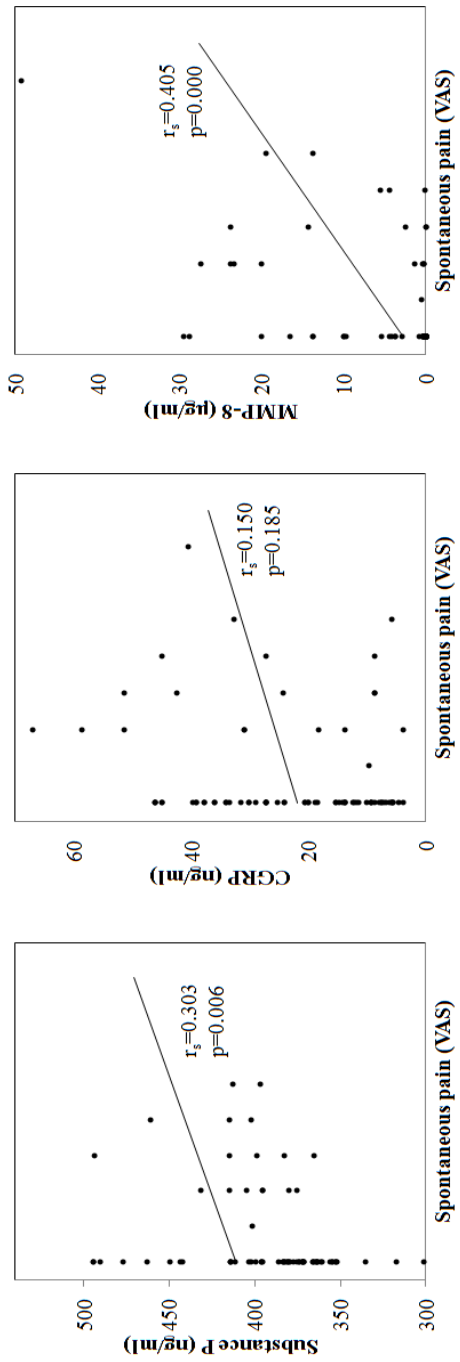


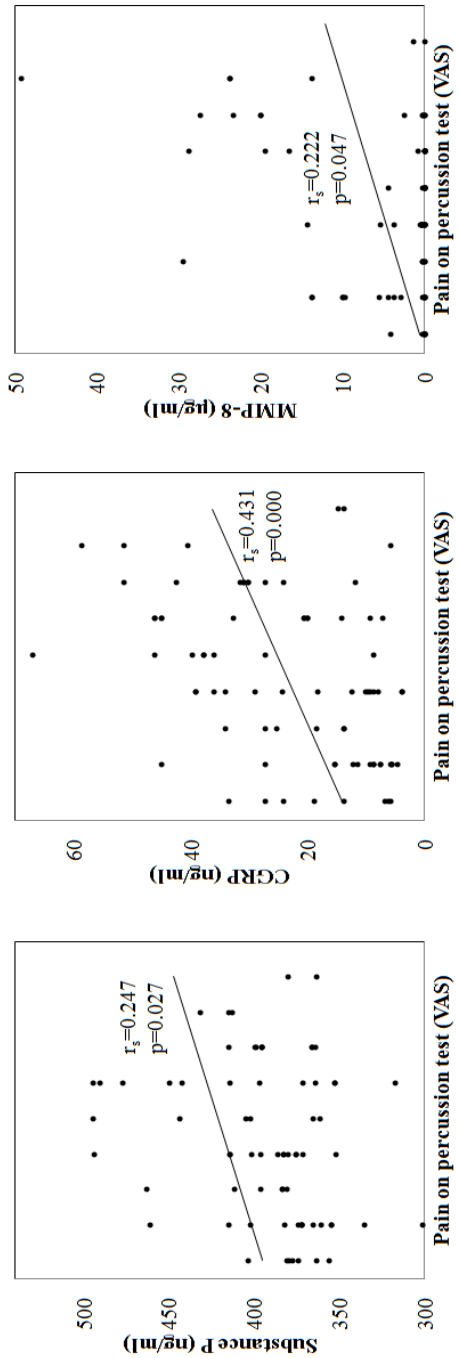
Figure 2. Box plots showing changes in concentrations of neuropeptides and inflammatory cytokine (%) after experimental treatments.

A


Substance P (ng/ml)
500
450
400
350
300
r_s=0.303
p=0.006
Spontaneous pain (VAS)
CGRP (ng/ml)
60
40
20
0
r_s=0.150
p=0.185
Spontaneous pain (VAS)
MMP-8 (ng/ml)
50
40
30
20
10
0
r_s=0.405
p=0.000
Spontaneous pain (VAS)

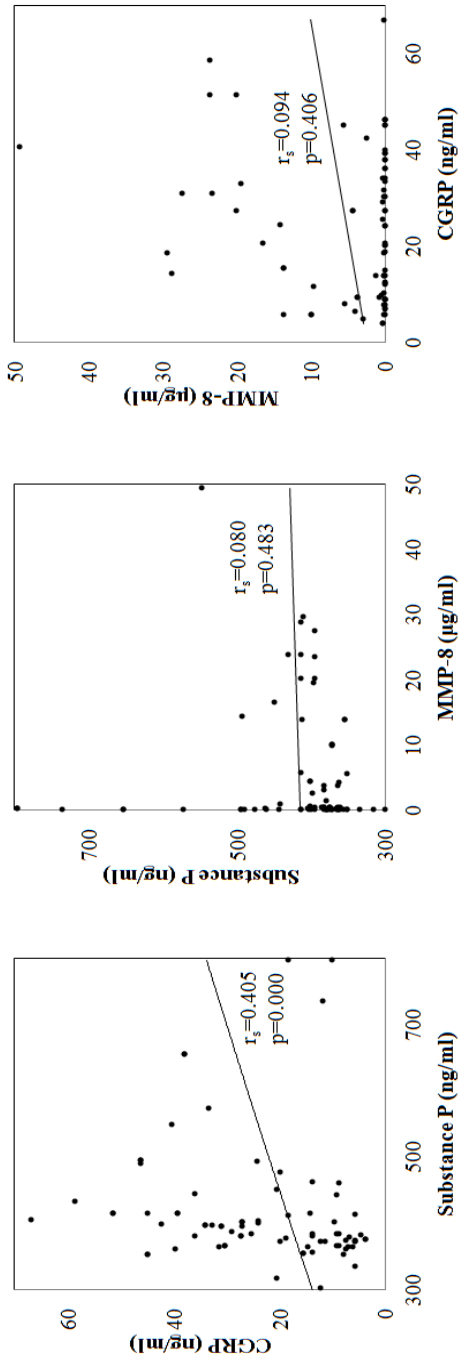
(continued)

B


MMP-8 (ng/ml)
0
10
20
30
40
50
r_s=0.222
p=0.047
Pain on percussion test (VAS)
CGRP (ng/ml)
0
20
40
60
r_s=0.431
p=0.000
Pain on percussion test (VAS)
Substance P (ng/ml)
300
350
400
450
500
r_s=0.247
p=0.027
Pain on percussion test (VAS)

(continued)

C


CGRP (ng/ml)
Substance P (ng/ml)
rs=0.405
p=0.000
Substance P (ng/ml)
MMP-8 (µg/ml)
rs=0.080
p=0.483
MMP-8 (µg/ml)
CGRP (ng/ml)
rs=0.094
p=0.406

(continued)

Figure 3. Scatter plots showing correlations between the degree of perceived pain and the levels of neuropeptides and inflammatory cytokine in root canal exudates. (A) Parameters correlated with spontaneous pain. (B) Parameters correlated with pain on percussion. (C) Correlations between SP, CGRP, and MMP-8. VAS, visual analogue scale (0, no pain; 10, extremely severe pain); SP, substance P; CGRP, calcitonin gene-related peptide; MMP, matrix metalloproteinase.

국문초록

1440-nm Nd:YAG 레이저가 지속적인 치근단 치주염을 가지는 치아의 동통 및 뉴로펩타이드/싸이토카인 감소에 미치는 영향에 대한 연구

유 연 지

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지도교수 백 승 호

목적

본 연구에서는 1440-nm Nd:YAG 레이저가 지속적인 치근단 치주염을 가지는 치아에서 동통 및 근관 내 삼출물에 포함되어 있는 뉴로펩타이드와 염증성 싸이토카인 수준 감소에 미치는 영향에 대해 알아보고자 하였다.

재료 및 방법

지속적인 근단성 치주염을 가지는 치아의 치료를 위해 의뢰된 40 명의 환자를 다음과 같이 두 개의 치료군으로 무작위 배정하여 치료하였다: group L,

통상적인 재근관 치료 과정에 부가적으로 1,440-nm Nd:YAG 레이저를 300-μm 지름의 광섬유 팁을 이용하여 근관 내에 조사함; group C, 통상적인 재근관 치료. 치료 전과 후에 자발통 및 타진시 동통의 수준을 visual analogue scale (VAS)를 이용하여 기록하고, 근관 내 삼출물을 채취하여 substance P (SP), calcitonin gene-related peptide (CGRP), 그리고 matrix metalloproteinase (MMP)-8 의 수준을 면역 검사법으로 정량 분석하였다.

각 치료군에서 치료 전과 후의 동통 변화 정도 (VAS 척도) 및 SP, CGRP, 그리고 MMP-8 수준 변화에 대해 유의성을 분석하고, 두 치료군 간의 차이를 비교하였다. 또한 환자가 느끼는 자발통 및 타진시 동통의 VAS 척도와 근관 내 삼출물의 SP, CGRP, 그리고 MMP-8 수준 사이의 상관 관계를 분석하였다. 유의 수준은 5%로 설정하였다.

결과

Group L에서는 치료 후 측정한 모든 척도들이 유의하게 감소하였고 ($p < .05$), group C에서는 타진시 동통, CGRP, 그리고 MMP-8의 수준이 유의하게 감소하였다 ($p < .05$). 지속적인 근단성 치주염을 가지는 치아의 재근관 치료에 1440-nm Nd:YAG 레이저를 부가적으로 조사한 경우 타진시 동통 및 SP, CGRP, 그리고 MMP-8 수준의 감소에 유의한 효과를 나타내었다 ($p < .05$). VAS로 표현한 환자가 느끼는 동통의 정도는 근관 내 삼출물에 포함된 동통 관련 뉴로펩타이드 및 염증성 싸이토카인의 수준과 유의한 양의 상관관계를 나타내었다 (p

< .05). SP 수준은 CGRP 수준과 유의한 양의 상관관계를 보였다 (p < .05).

결론

지속적인 근단성 치주염을 가지는 치아에 대한 재근관 치료에서 광섬유 팁을 이용한 1440-nm Nd:YAG 레이저의 적용은 동통 수준 감소 및 치근단 염증 완화에 효과적이었다.

주요어: Nd:YAG 레이저, 뉴로펩타이드, 동통, visual analogue scale, 광섬유 팁

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